Package 'NADfinder'

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Title Call wide peaks for sequencing data

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Description Nucleolus is an important structure inside the nucleus in eukaryotic cells. It is the site for transcribing rDNA into rRNA and for assembling ribosomes, aka ribosome biogenesis. In addition, nucleoli are dynamic hubs through which numerous proteins shuttle and contact specific non-rDNA genomic loci. Deep sequencing analyses of DNA associated with isolated nucleoli (NADseq) have shown that specific loci, termed nucleolusassociated domains (NADs) form frequent threedimensional associations with nucleoli. NAD-seq has been used to study the biological functions of NAD and the dynamics of NAD distribution during embryonic stem cell (ESC) differentiation. Here, we developed a Bioconductor package NADfinder for bioinformatic analysis of the NAD-seq data, including normalization, smoothing, peak calling, peak trimming and annotation.

License GPL (>= 2)

- **Depends** R (>= 3.4), BiocGenerics, IRanges, GenomicRanges, S4Vectors, SummarizedExperiment
- Imports graphics, methods, baseline, signal, GenomicAlignments, GenomeInfoDb, rtracklayer, limma, trackViewer, stats, utils, Rsamtools, metap, EmpiricalBrownsMethod,ATACseqQC, corrplot, csaw
- Suggests RUnit, BiocStyle, knitr, BSgenome.Mmusculus.UCSC.mm10, testthat, BiocManager

biocViews Sequencing, DNASeq, GeneRegulation, PeakDetection

LazyData TRUE

VignetteBuilder knitr

NADfinder-package

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NADfinder-package Identify nucleolus-associated domains (NADs) from NAD-seq

Description

Sliding-window based peak calling algorithm using whole genome sequences as control

backgroundCorrection Correct ratios for background

Description

Correct ratios of read counts per sliding window for background.

Usage

```
backgroundCorrection(ratios, degree = 3, ...)
```

Arguments

ratios	A vector of numeric. It is log2-transformed ratios, CPMRatios or OddRatios of counts for each window.
degree	Degree of polynomial. default 3.
	parameters could be passed to baseline.modpolyfit.

Details

This function implements the backgound correction methods of algorithm for polynomial fitting. See details via baseline.modpolyfit. This function expects the trendency of decreasing of the ratios from 5' end to 3' end.

Value

A vector of numeric. It is the background corrected log2-transformed ratios, CPMRatios or Odd-Ratios.

Examples

```
x <- runif(200)
background <- rep(c(20:1)/100, each=10)
backgroundCorrection(x)
```

butterFilter	Low pass filter on ratios	by butterworth filter

Description

The Butterworth filter is a type of signal processing filter designed to have as flat a frequency response as possible in the passband.

```
butterFilter(ratios, N = ceiling(length(ratios)/200))
```

ratios	A vector of numeric. It is log2-transformed ratios, CPMRatios or OddRatios in each window.
Ν	numeric(1) or integer(1). Critical frequencies of the low pass filter will be set as $1/N$. $1/N$ is a cutoff at $1/N$ -th of the Nyquist frequency. By default, it is suppose there are about 200 peaks in the inputs.

Value

A vector of numeric with same length of input ratios. The vector indicates smoothed ratios.

Examples

```
ratios <- runif(20000)
butterFilter(ratios)</pre>
```

callPeaks	Call peaks using transformed, background corrected, and smoothed
	ratios with biological replicates

Description

Use limma to calculate p-values for NADs

Usage

```
callPeaks(se, backgroundCorrectedAssay = "bcRatio",
normalization.method = "quantile", N = 100,
cutoffAdjPvalue = 1e-04, countFilter = 1000,
combineP.method = "minimump", smooth.method = "loess",
lfc = log2(1.5), ...)
```

Arguments

se	An object of RangedSummarizedExperiment with assays of raw counts, tran- formed ratios, background corrected ratios, smoothed ratios and z-scores. It should be an element of output of smoothRatiosByChromosome
backgroundCorr	rectedAssay
	character(1). Assays names for background corrected log2-transformed ratios,
	CPMRatios or OddRatios.
normalization.	method
	character(1) specifying the normalization method to be used. Choices are "none", "scale", "quantile" or "cyclicloess". See normalizeBetweenArrays for details.
Ν	numeric(1) or integer(1). The number of neighboring windows used for loess smoothing or the inverse of the critical frequencies of the low pass filter for butterworth filter. 1/N is a cutoff at 1/N-th of the Nyquist frequency. Default 100.
cutoffAdjPvalu	e
-	numeric(1). Cutoff adjust p-value.

countFilter	numeric(1). Cutoff value for mean of raw reads count in each window.
combineP.method	L L L L L L L L L L L L L L L L L L L
	A method used to combine P-values. Default minimump
<pre>smooth.method</pre>	A method used to smooth the ratios. Choices are "loess", "none" and "butter-worthfilter".
lfc	the minimum log2-fold-change that is considered scientifically meaningful
	Parameter not used.

Details

By default, use the mean smoothed ratio for each peak region to calculate p-values

Value

An object of GRanges of peak list with metadata "AveSig", "P.Value", and "adj.P.Val", where "AveSig" means average signal such as average log2OddsRatio, log2CPMRatio or log2Ratio.

Author(s)

Jianhong Ou, Haibo Liu and Julie Zhu

Examples

computeLibSizeChrom *Perform overlap queries between reads and genome by sliding windows Count reads over sliding windows.*

Description

Perform overlap queries between reads and genome by sliding windows Count reads over sliding windows.

```
computeLibSizeChrom(aln_list)
```

aln_list a list.

Value

A RangedSummarizedExperiment object with chromosome-level depth The assays slot holds the counts, rowRanges holds the annotation from the sliding widows of genome. metadata contains lib.size.chrom for holding chromosome-level sequence depth

Author(s)

Jun Yu, Hervé Pagès and Julie Zhu

cumulativePercentage Plot the cumulative percentage of tag allocation

Description

Plot the difference between the cumulative percentage of tag allocation in paired samples.

Usage

```
cumulativePercentage(se, binWidth = 1e+05,
backgroundCorrectedAssay = "bcRatio", ...)
```

Arguments

se	An object of RangedSummarizedExperiment with assays of raw counts, trans-	
	fomred ratios, background correct ratios, smoothed ratios and z-scores. It should	
	be an element of the output of smoothRatiosByChromosome.	
binWidth	numeric(1) or integer(1). The width of each bin.	
backgroundCorrectedAssay		
	character(1). Assays names for background correction ratios.	
	Parameter not used.	

Value

A list of data.frame with the cumulative percentages.

References

Normalization, bias correction, and peak calling for ChIP-seq Aaron Diaz, Kiyoub Park, Daniel A. Lim, Jun S. Song Stat Appl Genet Mol Biol. Author manuscript; available in PMC 2012 May 3.Published in final edited form as: Stat Appl Genet Mol Biol. 2012 Mar 31; 11(3): 10.1515/1544-6115.1750/j/sagmb.2012.11.issue-3/1544-6115.1750/1544-6115.1750.xml. Published online 2012 Mar 31. doi: 10.1515/1544-6115.1750 PMCID: PMC3342857

exportSignals

Examples

exportSignals Output signals for visualization

Description

Output signals to bedgraph, bed, wig, etc, for track viewer

Usage

```
exportSignals(dat, assayName, colName, con, format = "bedGraph", ...)
```

Arguments

dat	An object of GRanges, or RangedSummarizedExperiment with assays of raw counts, ratios, background correct ratios, smoothed ratios and z-scores. It should be an element of output of smoothRatiosByChromosome
assayName	character(1). Assay name for RangedSummarizedExperiment
colName	character(1). Column name of metadata of dat or assay of dat for coverage weight, see coverage, RangedSummarizedExperiment.
con	The connection to which data is saved. If this is a character vector, it is assumed to be a filename and a corresponding file connection is created and then closed after exporting the object. If missing, a SimpleRleList will be returned.
format	The format of the output. see export.
	Parameters to be passed to export

Value

If con is missing, a SimpleRleList will be returned. Otherwise, nothing is returned.

```
con="test.bedGraph", trackLine=myTrackLine)
data(triplicate.count)
exportSignals(triplicate.count, "counts",
                                "G18.subsampled.srt.bam", "test.bw", format="bigWig")
```

getCorrelations Get correlation coefficinets and p-values between biological replicates

Description

Get correlations and p-values between biological replicates based on coverage signal for peak regions. The signals will be filtered by the background cutoff value before calculated correlations. This function also output a correlation plots using the corrplot.

Usage

```
getCorrelations(se, chr = paste0("chr", seq_len(19)),
ratioAssay = "ratio", window = 10000L, cutoff = 1,
method = c("spearman", "pearson", "kendall"),
file_name = "Correlation plots.pdf", ...)
```

Arguments

se	A RangedSummarizedExperiment object. The output from log2se.
chr	A vector of character. Filter for seqnames. It should be the chromosome names to be kept.
ratioAssay	character(1). Column name of ratio for correlation calculation.
window	numeric(1) or integer(1). The window size for summary of the ratios.
cutoff	numeric(1). All the coverage signals lower than cutoff value in a given window will be filtered out.
method	character(1) indicating which correlation coefficient is to be computed. See cor.
file_name	A file name for output correlation plots
	Parameters not used.

Value

A list of matrixes of correlation coefficients and p-values.

Author(s)

Jianhong Ou, Haibo Liu

groupZscores

Examples

groupZscores

Calculate z-scores for each peak

Description

Detect peaks and calculate z-scores for each peak

Usage

```
groupZscores(zscore)
```

Arguments

zscore A vector of numeric. It is the z-scores of ratios for each window.

Value

A data.frame with column names as "zscore", "group", "grp.zscore", and "pvalue".

```
x <- seq_len(500)
a <- 2 * 2*pi/length(x)
y <- 20 * sin(x*a)
noise1 <- 20 * 1/10 * sin(x*a*10)
zscore <- y+noise1
groupZscores(zscore)</pre>
```

IntersectionNotStrict Count reads overlapping genomic ranges

Description

Count reads overlapping a set of genime features represented as genomic ranges. This function does not work for parallel.

Usage

```
IntersectionNotStrict(features, reads, ignore.strand = TRUE,
    inter.feature = FALSE)
```

Arguments

features	A object of GRanges representing the feature regions to be counted.
reads	An object that represents the data to be counted. See summarizeOverlaps. If reads are more than 1 bam files, it should be a vector of character with full path, otherwise current working directory is the default directory. For paired end reads,
ignore.strand	logical(1). ignore strand?
inter.feature	not used. This parameter is required by summarizeOverlaps.

Value

return a summarized experiment object with chromosome-level depth information for each input sample as metadata.

log2se

calculate the log2 transformed ratios for SummarizedExperiment class

Description

Calculate the log2 transformed ratios for nucleolus vs genome. pseudo-count will be used to avoid x/0 or log(0).

```
log2se(se, nucleolusCols, genomeCols, pseudocount = 1L,
transformation = c("log2OddsRatio", "log2CPMRatio", "log2Ratio"),
chrom.level.lib = TRUE)
```

peakdet

Arguments

se	A RangedSummarizedExperiment object. The output of tileCount.
nucleolusCols,	genomeCols
	column Names of counts for nucleolus and genome. They should be the col- umn names in the assays of se. Ratios will be calculated as log2(transformed nucleolusCols/transformed genomeCols).
pseudocount	default to 1, pseudo-count used to avoid $x/0$ or $log(0)$.
transformation	transformation type
chrom.level.lib	
	indicating whether calculating CPM or odds using sequence depth of the whole genome or the corresponding chromosome

Value

A RangedSummarizedExperiment object with log2 transformed ratios. Assays will be named as nucleolus, genome and ratio.

Author(s)

Jianhong Ou and Julie Zhu

Examples

peakdet

Detect peak positions

Description

Detect the peak positions and valley positions leveraging github::dgromer/peakdet

Usage

peakdet(y, delta = 0, silence = TRUE)

У	A numeric vector for searching peaks
delta	A numeric vector of length 1, defining the minimum absolute changes required for local maximum or minimum detection when slope sign changes. If it is set to 0, the delta will be set to 1/10 of the range of y.
silence	logical(1). If false, echo the delta value when delta is set as 0.

Value

A list with peakpos and valleypos. Both peakpos and valleypos are numeric vectors storing the positions of peaks or valleys.

Examples

```
y <- runif(200)
peakdet(y)
y <- sin(seq(0,20))
peakdet(y)</pre>
```

```
plotSig
```

Plot signals with ideograms

Description

Plot signals with ideograms for GRangesList.

Usage

```
plotSig(ideo, grList, mcolName, ...)
```

Arguments

ideo	Output of loadIdeogram.
grList	A GRangesList of data to plot.
mcolName	Column name of metadata of GRangesList for plotting.
	Parameters to pass to ideogramPlot

Value

Invisible argument list for ideogramPlot.

single.count

```
grList <- GRangesList(gr1, gr2)
plotSig(ideo, grList, mcolName="score", layout=list("chr1"))</pre>
```

single.count

Counts data for chromosome 18 for an experiment of a single pair of samples

Description

Counts data for chromosome 18 for an experiment of a single pair of samples

smoothRatiosByChromosome

Backgound correction and signal smoothing per chromosome

Description

Split the ratios by chromosome and do background correction and signal smoothing.

Usage

```
smoothRatiosByChromosome(se, chr = paste0("chr", c(seq_len(21), "X",
"Y")), ratioAssay = "ratio", backgroundCorrectedAssay = "bcRatio",
smoothedRatioAssay = "smoothedRatio", zscoreAssay = "zscore",
backgroundPercentage = 0.25, chrom.level.background = TRUE, ...)
```

Arguments

se	An object of RangedSummarizedExperiment with log2-transformed ratios, CPM- Ratios or OddRatios. Output of log2se
chr	A character vector, used to filter out seqnames. It should be the chromosome names to be kept.
ratioAssay	The name of assay in se, which store the values (log2-transformed ratios, CPM-Ratios or OddRatios) to be smoothed.
backgroundCorrectedAssay, smoothedRatioAssay, zscoreAssay	
	character(1). Assays names for background corrected ratios, smoothed ratios and z-scores based on background corrected ratios.
backgroundPercentage	
	numeric(1). Percentage of values for background, see zscoreOverBck. The per- centage of values lower than this threshold will be treated as background, with 25 percentile as default.
chrom.level.background	
	logical(1): TRUE or FALSE, default to TRUE, use chromosome-level back- ground to calculate z-score
	Parameters could be passed to butterFilter.

Value

A SimpleList of RangedSummarizedExperiment with smoothed ratios.

Author(s)

Jianhong Ou, Haibo Liu and Julie Zhu

Examples

```
tileCount
```

Perform overlap queries between reads and genome by windows

Description

tileCount extends summarizeOverlaps by finding coverage for each fixed window in the whole genome

Usage

```
tileCount(reads, genome, excludeChrs = c("chrM", "M", "Mt", "MT"),
windowSize = 50000, step = 10000, mode = IntersectionNotStrict,
dataOverSamples = FALSE, ...)
```

Arguments

reads	A GRanges, GRangesList (should be one read per list element), GAlignments, GAlignmentsList, GAlignmentPairs or BamFileList object that represents the data to be counted by summarizeOverlaps. If reads are more than 1 bam files, it should be a vector of character with full path, otherwise current working directory is the default directory.
genome	A BSgenome object from/on which to get/set the sequence and metadata infor- mation.
excludeChrs	A vector of string: chromosomes/scaffolds of no interest for NAD analysis. see summarizeOverlaps. default is countByOverlaps, alia of countOverlaps(features, reads, ignore.strand=ignore.strand)
windowSize	numeric(1) or integer(1). Size of the windows.
step	numeric(1) or integer(1). Step of generating silding windows.
mode	One of the pre-defined count methods.
dataOverSamples	
	logical(1). Data over several samples when use GRangesList as input.
	Additional arguments passed to summarizeOverlaps.

tileCount2

Value

A RangedSummarizedExperiment object. The assays slot holds the counts, rowRanges holds the annotation from the sliding widows of genome. metadata contains lib.size.chrom for holding chromosome-level sequence depth

Author(s)

Jianhong Ou, Haibo Liu, Herve Pages and Julie Zhu

Examples

```
if (interactive())
{
    fls <- list.files(system.file("extdata", package="NADfinder"),</pre>
    recursive=FALSE, pattern="*bam$", full=TRUE)
    names(fls) <- basename(fls)</pre>
    if (!require(BSgenome.Mmusculus.UCSC.mm10))
    {
        if (!requireNamespace("BiocManager", quietly=TRUE))
        install.packages("BiocManager")
        BiocManager::install("BSgenome.Mmusculus.UCSC.mm10")
        library(BSgenome.Mmusculus.UCSC.mm10)
    }
    se <- tileCount(reads = fls,</pre>
                    genome = Mmusculus,
                    excludeChrs = c("chrM", paste0("chr", c(1:17,19)),
                                      "chrX", "chrY"),
                    windowSize=50000, step=10000)
}
```

leCount2	Perform overlap queries between reads and genome by sliding win-
	dows Count reads over sliding windows.

Description

ti

Perform overlap queries between reads and genome by sliding windows Count reads over sliding windows.

if (interactive())

fls <- list.files(system.file("extdata", package="NADfinder"), recursive=FALSE, pattern="*bam\$", full=TRUE) names(fls) <- basename(fls)

se <- tileCount2(reads = fls, windowSize=50000, step=10000)

```
tileCount2(reads, fragment.length = 100, windowSize = 50000,
  restrict = paste0("chr", c(1:19, "X", "Y")), step = 1000,
  filter = 0, pe = "both")
```

```
tileCount2(reads, fragment.length = 100, windowSize = 50000,
  restrict = paste0("chr", c(1:19, "X", "Y")), step = 1000,
  filter = 0, pe = "both")
```

reads	An object that represents the names and path of the bam files to be counted. If reads are more than 1 bam files, it should be a vector of character with full path. This function now works for paired end reads
fragment.lengt	h
	integer(1). An integer scalar or a list of two integer scalars/vectors, containing the average length(s) of the sequenced fragments in each libary.
windowSize	numeric(1) or integer(1). Size of the windows.
restrict	restrict to a set of chromosomes, default to mouse chromosomes.
step	numeric(1) or integer(1). Step of generating silding windows.
filter	default to 0 without filtering. An integer scalar for the minimum count sum across libraries for each window
ре	a character string indicating whether paired-end data is present; set to "none", "both", "first" or "second"

Value

A RangedSummarizedExperiment object with chromosome-level depth The assays slot holds the counts, rowRanges holds the annotation from the sliding widows of genome. metadata contains lib.size.chrom for holding chromosome-level sequence depth

Author(s)

Jun Yu, Hervé Pagès and Julie Zhu

Examples

transformData

. .

Description

calculate the log2 ratios, log2 cpm (count per million) ratios, or log2 odds ratios for nucleolus vs genome. pseudo-count will be used to avoid x/0 or log(0).

Usage

```
transformData(A, B, seqnames.A, seqnames.B, pseudo.count = 1L,
transformation = c("log2OddsRatio", "log2CPMRatio", "log2Ratio"),
chrom.level.lib = TRUE, lib.size.A, lib.size.B)
```

. .

Arguments

А, В	window-level counts for nucleolus and genome, extracted from the assays of the output of the tileCounts function
seqnames.A, seqnames.B	
	seqnames, extracted from the rowRanges of the ouput of the tileCounts function
pseudo.count	pseudo-count will be used to aviod x/0 or log0, defult to 1.
transformation	transformation type
chrom.level.lib	
	indicating whether calculating CPM or odds using sequence depth of the whole genome or the corresponding chromosome
lib.size.A, lib.size.B	
	library size for A and B. these two dataframes contain chromosome-level se- quence depth for the chromosomes, which can be extracted from the metadata of the output of the tileCounts function

. .

Value

a numeric vector of log2 ratios, log2 CPM ratios or log2 odds ratios.

Author(s)

Julie Zhu

Examples

```
transformData(seq_len(10), 10:1, seqnames.A = Rle(c("chr1", "chr2"), c(5,5)),
Rle(c("chr1", "chr2"), c(5,5)), transformation = "log2OddsRatio",
chrom.level.lib = FALSE, lib.size.A = cbind(c("chr1", "chr2"), c(10000, 12000)),
lib.size.B = cbind(c("chr1", "chr2"), c(10000, 12000)))
transformData(seq_len(10), 10:1, seqnames.A = Rle(c("chr1", "chr2"), c(5,5)),
Rle(c("chr1", "chr2"), c(5,5)), transformation = "log2CPMRatio",
chrom.level.lib = FALSE, lib.size.A = cbind(c("chr1", "chr2"), c(10000, 12000)),
lib.size.B = cbind(c("chr1", "chr2"), c(10000, 12000)))
transformData(seq_len(10), 10:1, seqnames.A = Rle(c("chr1", "chr2"), c(5,5)),
Rle(c("chr1", "chr2"), c(5,5)), transformation = "log2CPMRatio",
chrom.level.lib = TRUE, lib.size.A = cbind(c("chr1", "chr2"), c(5,5)),
Rle(c("chr1", "chr2"), c(5,5)), transformation = "log2CPMRatio",
chrom.level.lib = TRUE, lib.size.A = cbind(c("chr1", "chr2"), c(1000, 12000)),
```

. .

trimPeaks

```
lib.size.B = cbind(c("chr1", "chr2"), c(10000, 200)))
transformData(seq_len(10), 10:1, seqnames.A = Rle(c("chr1", "chr2"), c(5,5)),
Rle(c("chr1", "chr2"), c(5,5)), transformation = "log20ddsRatio",
chrom.level.lib = TRUE, lib.size.A = cbind(c("chr1", "chr2"), c(100, 12000)),
lib.size.B = cbind(c("chr1", "chr2"), c(10000, 200)))
transformData(seq_len(10), 10:1, transformation = "log2Ratio")
```

trimPeaks

Trim peaks

Description

Filter the peaks by pvalue and trim the range of peaks for an NAD experiment without biological replicates.

Usage

```
trimPeaks(se, cutoffPvalue = 0.05, backgroundPercentage = 0.25,
  countFilter = 1000, ratioAssay = "ratio",
  backgroundCorrectedAssay = "bcRatio",
  smoothedRatioAssay = "smoothedRatio", zscoreAssay = "zscore")
```

Arguments

se	An object of RangedSummarizedExperiment with assays of raw counts, ratios, background corrected ratios, smoothed ratios and z-scores. It should be an element of the output of smoothRatiosByChromosome
cutoffPvalue	numeric(1). Cutoff p-value.
backgroundPercentage	
	numeric(1). Cutoff value for the peaks height.
countFilter	numeric(1) or integer(1). Cutoff value for mean of raw reads count in each window.
ratioAssay	character(1). The name of assay in se, which store the values to be smoothed.
backgroundCorrectedAssay, smoothedRatioAssay, zscoreAssay	
	Assays names for background-corrected ratios, smoothed ratios and z-scores based on background corrected ratios.

Value

An object of GRanges.

Examples

```
data(single.count)
se <- single.count
dat <- log2se(se, nucleolusCols="N18.subsampled.srt.bam", genomeCols="G18.subsampled.srt.bam",
transformation="log2CPMRatio")
## Smooth the ratios for each chromosome.
dat <- smoothRatiosByChromosome(dat, N=100, chr=c("chr18","chr19"))
peaks <- trimPeaks(dat[["chr18"]],</pre>
```

triplicate.count

backgroundPercentage=.25, cutoffPvalue=0.05, countFilter=1000)

triplicate.count Counts data for chromosome 18 for an expriment with triplicates

Description

Counts data for chromosome 18 for an expriment with triplicates

zscoreOverBck Z-scores over the background

Description

Calculate the z-scores over the background distribution.

Usage

zscoreOverBck(ratios, backgroundPercentage = 0.25)

Arguments

ratios A numeric vector containing the transformed, background corrected and smoothed ratios in each window. backgroundPercentage numeric(1). Low percentile for background distribution.

Value

A vector of numeric. Z-scores.

Author(s)

Jianhong Ou and Julie Zhu

```
r <- runif(200)
zscoreOverBck(r)</pre>
```

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